

WHAT IS CLAIMED IS:

Sub 21
1 1. A method of extracting structural information from a NMR data set for
2 a selected macromolecule in an intact biological compartment wherein said selected
3 macromolecule is labeled with an NMR-detectable nucleus, such that said nucleus is present
4 in said macromolecule in an amount greater than is naturally abundant in said
5 macromolecule, said method comprising:

6 (a) contacting said cell with radio frequency energy, thereby producing an excited
7 NMR-detectable nucleus;

8 (b) collecting radio frequency data from said excited NMR-detectable nucleus,
9 thereby producing said NMR data set, and

10 (c) analyzing said data set to extract said structural information for said selected
11 macromolecule from said data set.

1 2. The method according to claim 1, wherein said selected
2 macromolecule is overexpressed in said biological compartment.

1 3. The method according to claim 1, wherein said NMR-detectable
2 nucleus is present in an amount detectable by NMR of said biological compartment.

1 4. The method according to claim 1, wherein said selected
2 macromolecule is a member selected from the group consisting of proteins, saccharides,
3 glycoproteins, and nucleic acids.

1 5. The method according to claim 1, wherein said selected
2 macromolecule is in a complex with a small molecule.

1 6. The method according to claim 5, wherein said small molecule is an
2 exogenous small molecule.

1 7. The method according to claim 5, wherein said small molecule is a
2 therapeutic agent or a candidate therapeutic agent.

1 8. The method according to claim 7, wherein said small molecule is an
2 exogenous small molecule.

030543.07.13.1
TDE 20.6E45660

1 9. The method according to claim 1, wherein said macromolecule is
2 further labeled with deuterium.

1 10. The method according to claim 1, wherein said biological compartment
2 is present in a suspension.

1 11. The method according to claim 1, wherein said structural information
2 is conformational information.

1 12. The method according to claim 1, wherein said structural information
2 is for a complex formed between said selected macromolecule and a small molecule selected
3 from therapeutic agents and candidate therapeutic agents.

1 13. The method according to claim 1, wherein said structural information
2 is for a complex formed between said selected macromolecule and a member selected from
3 small molecules, endogenous macromolecules and combinations thereof.

1 14. The method according to claim 1, wherein said structural information
2 is for a first conformation of said selected macromolecule and a second conformation of said
3 selected macromolecule.

1 15. The method according to claim 1, wherein said data set is acquired by
2 a triple resonance NMR method.

1 16. The method according to claim 15, wherein said triple resonance NMR
2 experiment is a member selected from HSQC and TROSY.

1 17. The method according to claim 1, wherein said biological compartment
2 is prepared by a method comprising:

- 3 (a) transforming an unlabeled precursor of said labeled biological compartment with
4 a nucleic acid encoding said selected macromolecule, wherein said nucleic
5 acid is operably linked to a promoter non-native to said unlabeled precursor
6 cell, thereby producing a transformed biological compartment;
7 (b) incubating said transformed biological compartment in a medium comprising said
8 NMR-detectable nucleus; and

9 (c) inducing said transformed biological compartment, thereby preparing said labeled
10 biological compartment.

1 18. The method according to claim 17, further comprising:
2 (d) inhibiting essentially all transcription in said transformed biological compartment,
3 which is under control of promoters native to said unlabeled precursor
4 biological compartment, while allowing transcription under control of said
5 non-native promoter to proceed.

1 19. The method according to claim 17, wherein said medium comprises an
2 amino acid labeled with said NMR sensitive nucleus.

1 20. The method according to claim 17, wherein said medium is deuterated.

1 21. The method according to claim 17, wherein said biological
2 compartment is a bacterial cell.

1 22. The method according to claim 17, wherein the non-native promoter
2 encodes an RNA polymerase that is operable during step (d).

1 23. The method according to claim 17, wherein the non-native promoter is
2 a phage promoter.

1 24. The method according to claim 18, wherein said inhibiting is caused by
2 administering an inhibitor to said biological compartment in an amount sufficient to cause
3 said inhibiting.

1 25. The method according to claim 24, wherein said inhibitor is rifampicin.

1 26. The method of claim 1, wherein said selected macromolecule
2 experiences a local viscosity at least 2 fold greater than the viscosity of pure water, wherein
3 said local viscosity and said viscosity of said pure water are determined at the same
4 temperature.

1 27. The method of claim 1, wherein said selected macromolecule is
2 present in said biological compartment at a weight percent of up to 0.3% compared to the
3 total weight of said biological compartment.

1 28. The method of claim 1, wherein said selected macromolecule is
2 present in said biological compartment at a weight percent of up to 50% compared to the total
3 weight of said biological compartment.

1 29. The method of claim 1, wherein said selected macromolecule has a
2 molecular weight of at least 5 kDa.

1 30. The method of claim 1, wherein said selected macromolecule has a
2 molecular weight of at least 25 kDa.

1 31. The method of claim 1, wherein said selected macromolecule has a
2 molecular weight of at least 70 kDa.

1 32. The method of claim 1, wherein said biological compartment is a
2 living cell.

1 33. The method of claim 1, wherein said biological compartment is a cell
2 that has been metabolically arrested.

1 34. The method of claim 1, wherein said selected macromolecule is
2 expressed from a plasmid.

1 35. The method of claim 1, using a multidimensional multinuclear method.

1 36. The method of claim 35, using an HNCA experiment.

1 37. The method of claim 35, using an HMQC experiment.

1 38. The method of claim 1, wherein said compartment is a biological cell.

1 39. The method of claim 38, wherein said cell is a prokaryotic cell.

1 40. The method of claim 39, wherein said cell is a *E. coli* cell.

1 41. The method of claim 38, wherein said cell is a eukaryotic cell.

1 42. The method of claim 41, wherein said cell is a yeast cell.

1 43. The method of claim 41, wherein said cell is a mammalian cell.

009065439-071304
FOETZD-6E450660

- 1 44. The method of claim 43, wherein said cell is a human cell.
- 1 45. A method of extracting structural information from a NMR data set for
2 a selected macromolecule of an intact biological compartment wherein said selected
3 macromolecule is labeled with a NMR-detectable nucleus, such that said nucleus is present in
4 said macromolecule in an amount greater than is naturally abundant in said macromolecule,
5 wherein said nucleus is not ¹⁹F, said method comprising:
6 (a) contacting said biological compartment with radio frequency energy,
7 thereby producing an excited NMR-detectable nucleus, and
8 (b) collecting radio frequency data from said excited NMR-detectable
9 nucleus, thereby producing said NMR data set.
- 1 46. The method according to claim 45, wherein said selected
2 macromolecule is overexpressed in said biological compartment.
- 1 47. The method according to claim 45, wherein said NMR-detectable
2 nucleus is present in an amount detectable by NMR of said intact, biological compartment.
- 1 48. The method according to claim 45, wherein said selected
2 macromolecule is a member selected from the group consisting of proteins, saccharides,
3 glycoproteins, and nucleic acids.
- 1 49. The method according to claim 45, wherein said selected
2 macromolecule is in a complex with a small molecule.
- 1 50. The method according to claim 49, wherein said small molecule is an
2 exogenous small molecule.
- 1 51. The method according to claim 49, wherein said small molecule is a
2 therapeutic agent or a candidate therapeutic agent.
- 1 52. The method according to claim 51, wherein said small molecule is an
2 exogenous small molecule.
- 1 53. The method according to claim 45, wherein said macromolecule is
2 further labeled with deuterium.

09905439.071301
FOET 20 65450660

1 **54.** The method according to claim **45**, wherein said biological
2 compartment is present in a suspension.

1 **55.** The method according to claim **45**, wherein said structural information
2 is conformational information.

1 **56.** The method according to claim **45**, wherein said structural information
2 is for a complex formed between said selected macromolecule and a small molecule selected
3 from therapeutic agents and candidate therapeutic agents.

1 **57.** The method according to claim **45**, wherein said structural information
2 is for a complex formed between said selected macromolecule and a member selected from
3 small molecules, endogenous macromolecules and combinations thereof.

1 **58.** The method according to claim **45**, wherein said structural information
2 is for a first conformation of said selected macromolecule and a second conformation of said
3 selected macromolecule.

1 **59.** The method according to claim **45**, wherein said data set is acquired by
2 a triple resonance NMR method.

1 **60.** The method according to claim **59**, wherein said triple resonance NMR
2 experiment is a member selected from HSQC and TROSY.

1 **61.** The method according to claim **45**, wherein said biological
2 compartment is prepared by a method comprising:

3 (a) transforming an unlabeled precursor of said labeled biological compartment with
4 a nucleic acid encoding said selected macromolecule, wherein said nucleic
5 acid is operably linked to a promoter non-native to said unlabeled precursor
6 biological compartment, thereby producing a transformed biological
7 compartment;

8 (b) incubating said transformed biological compartment in a medium comprising said
9 NMR-detectable nucleus; and

10 (c) inducing said transformed biological compartment, thereby preparing said labeled
11 biological compartment.

1 **62.** The method according to claim **61**, further comprising:
2 (d) inhibiting essentially all transcription in said transformed biological compartment,
3 which is under control of promoters native to said unlabeled precursor
4 biological compartment, while allowing transcription under control of said
5 non-native promoter to proceed.

1 **63.** The method according to claim **61**, wherein said medium comprises an
2 amino acid labeled with said NMR sensitive nucleus.

1 **64.** The method according to claim **61**, wherein said medium is deuterated.

1 **65.** The method according to claim **61**, wherein said biological
2 compartment is a bacterial cell.

1 **66.** The method according to claim **61**, wherein the non-native promoter
2 encodes an RNA polymerase that is operable during step (d).

1 **67.** The method according to claim **61**, wherein the non-native promoter is
2 a phage promoter.

1 **68.** The method according to claim **62**, wherein said inhibiting is caused by
2 administering an inhibitor to said biological compartment in an amount sufficient to cause
3 said inhibiting.

1 **69.** The method according to claim **68**, wherein said inhibitor is rifampicin.

1 **70.** The method of claim **45**, wherein said selected macromolecule
2 experiences a local viscosity at least 2 fold greater than the viscosity of pure water, wherein
3 said local viscosity and said viscosity of said pure water are determined at the same
4 temperature.

1 **71.** The method of claim **45**, wherein said selected macromolecule is
2 present in said biological compartment at a weight percent of up to 0.3% compared to the
3 total weight of said biological compartment.

006605450660
TDE120"66450660

1 72. The method of claim 45, wherein said selected macromolecule is
2 present in said biological compartment at a weight percent of up to 50% compared to the total
3 weight of said biological compartment.

1 73. The method of claim 45, wherein said selected macromolecule has a
2 molecular weight of at least 5 kDa.

1 74. The method of claim 45, wherein said selected macromolecule has a
2 molecular weight of at least 25 kDa.

1 75. The method of claim 45, wherein said selected macromolecule has a
2 molecular weight of at least 70 kDa.

1 76. The method of claim 45, wherein said biological compartment is a
2 living cell.

1 77. The method of claim 45, wherein said biological compartment is a cell
2 that has been metabolically arrested.

1 78. The method of claim 45, wherein said selected macromolecule is
2 expressed from a plasmid.

1 79. The method of claim 45, using a multidimensional multinuclear
2 method.

1 80. The method of claim 79, using an HNCA experiment.

1 81. The method of claim 79, using an HMQC experiment.

1 82. The method of claim 45, wherein said compartment is a biological cell.

1 83. The method of claim 82, wherein said cell is a prokaryotic cell.

1 84. The method of claim 83, wherein said cell is a *E. coli* cell.

1 85. The method of claim 83, wherein said cell is a eukaryotic cell.

1 86. The method of claim 85, wherein said cell is a yeast cell.

87. The method of claim **85**, wherein said e cell is a mammalian cell.

88. The method of claim 87, wherein said cell is a human cell.

37